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Regulation of bacterial sugar–H^+ symport by phospho*enol*pyruvate-dependent enzyme I/HPr-mediated phosphorylation

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ABSTRACT **The lactose–H^+ symport protein (LacS) of *Streptococcus thermophilus* has a C-terminal hydrophilic domain that is homologous to IIA protein(s) domains of the phospho*enol*pyruvate:sugar phosphotransferase system (PTS). C-terminal truncation mutants were constructed and expressed in *Escherichia coli* and their properties were analyzed. Remarkably, the entire IIA domain (160 amino acids) could be deleted without significant effect on lactose–H^+ symport and galactoside equilibrium exchange. Analysis of the LacS mutants in *S. thermophilus* cells suggested that transport is affected by PTS-mediated phosphorylation of the IIA domain. For further studies, membrane vesicles of *S. thermophilus* were fused with cytochrome *c* oxidase-containing liposomes, and, when appropriate, phospho*enol*pyruvate (PEP) plus purified enzyme I and heat-stable protein HPr were incorporated into the hybrid membranes. Generation of a protonmotive force (Δp) in the hybrid membranes resulted in accumulation of lactose, whereas uptake of the PTS sugar sucrose was not observed. With PEP and the energy-coupling proteins enzyme I and HPr of the PTS on the inside, high rates of sucrose uptake were observed, whereas Δp-driven lactose uptake by wild-type LacS was inhibited. This inhibition was not observed with LacS(Δ160) and LacS(H552R), indicating that PEP-dependent enzyme I/HPr-mediated phosphorylation of the IIA domain (possibly the conserved His-552 residue) modulates lactose–H^+ symport activity.**

The phospho*enol*pyruvate:sugar phosphotransferase system (PTS) catalyzes phosphoryl transfer from phospho*enol*pyruvate (PEP) to sugars (e.g., glucose) via a number of energy-coupling proteins—i.e., enzyme I, heat-stable protein HPr, IIA, and IIB (1). In addition to catalyzing sugar transport, the PTS is involved in regulation of non-PTS transport, carbon and nitrogen metabolism, chemotaxis, and other processes (1–3).

In the Gram-negative enteric bacteria, transport of sugars can be regulated at the level of the transport enzyme itself (inducer exclusion) but also at the level of protein expression (induction, catabolite repression) (1, 3). This dual regulation allows an instantaneous response of the organism to the presence or absence of a specific sugar and a slow response, which involves switching on/off the transcription of certain genes. The PTS has a central role in this regulation since the phosphorylation state of the phosphoryl transfer protein IIA^{Glc} affects the activity of various non-PTS transport enzymes (inducer exclusion) as well as cAMP synthesis (catabolite repression). The phosphorylation state of IIA^{Glc} is determined by the balance between phosphorylation via HPr~P and dephosphorylation via $IICB^{Glc}$ in the presence of substrate (e.g., glucose). The result of this regulation is that when *Escherichia coli* grows in the presence of glucose (PTS sugar) and a non-PTS sugar like lactose or melibiose, diauxic growth is observed with glucose being used first (1, 3).

The involvement of IIA^{Glc} or IIA-like proteins in PTS-mediated regulation in nonenteric bacteria—e.g., Gram-positives—is unclear. The surprising observation has been made, however, that a number of non-PTS sugar transport proteins have a C-terminal extension that is homologous to IIA^{Glc} of *E. coli* (4). The best-characterized system of this family of transport proteins with a two-domain structure is the lactose transport protein (LacS) of *Streptococcus thermophilus* (4–8). The LacS protein catalyzes the uptake of galactosides in symport with a proton or exchanges lactose for intracellularly formed galactose (6, 7). The N-terminal (carrier) domain of LacS is typical for a polytopic membrane protein and is composed of 12 α-helical transmembrane segments; the C-terminal IIA domain is hydrophilic and ≈160 amino acids in size (4). It has been suggested that structurally and functionally distinct domains such as the carrier and IIA part of LacS are connected by interdomain structures (or Q-linkers) that allow the different domains to interact functionally (9).

In the present study, we demonstrate that LacS is the only galactoside transporting activity in *S. thermophilus* and that deletion of the LacS IIA domain relieves the transport from inhibition by a PTS sugar. Using an artificial membrane system, in which Δp-driven lactose uptake and PTS-mediated sucrose uptake activities were reconstituted, we show that lactose transport is inhibited by PEP-dependent enzyme I/HPr-mediated phosphorylation of the IIA domain of LacS.

MATERIALS AND METHODS

Strains and Growth Conditions. *E. coli* strains were grown aerobically at 37°C in Luria broth (10) unless specified otherwise. The *E. coli* strains used were DW2 [*lacI*$^+$, Δ*lac*(*ZY*), *melA*$^+$, Δ*melB*, *strA*] and HB101 [*hsdS20*($r_B^-m_B^-$), *recA13*, *ara14*, *proA2*, *lacY1*, *galK2*, *rps*(Sm^R), *xyl-5*, *mtl-1*, *supE44*, λ^-, F^-]. Strains carrying the plasmids pSKE8 or derivatives were grown in medium supplemented with carbenicillin (50 μg/ml) (8). *S. thermophilus* ST11 (wild type) and deletion derivatives Δ*lacS* and Δ*lacZ* were grown semianaerobically at 42°C in Elliker broth (11) containing 0.5% beef extract (Belliker medium) and supplemented with 20 mM lactose (or 20 mM sucrose plus 20 mM galactose—i.e., when the strains are Lac^-). Erythromycin (5 μg/ml) was added to the medium when the *Streptococcus* strains carried pGK13, pGKGS8, or derivatives (see below).

Isolation of Membrane Vesicles and Membrane Fusion. Membrane vesicles of *S. thermophilus* and cytochrome *c* oxidase-containing liposomes were prepared as described (7). Fusion between membrane vesicles and cytochrome *c* oxidase-containing liposomes was performed by freeze/thaw sonica-

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Abbreviations: PTS, phospho*enol*pyruvate:sugar phosphotransferase system; PEP, phospho*enol*pyruvate; TMPD, *N,N,N',N'*-tetramethylphenylenediamine; TMG, methyl β-D-thiogalactopyranoside.

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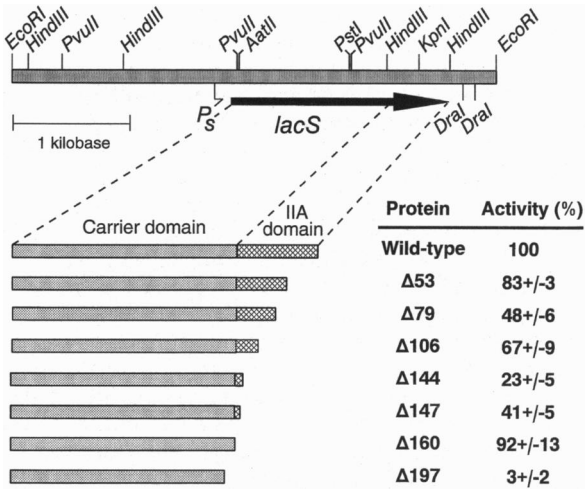
tion (12). Briefly, the proteoliposomes (20 mg of phospholipid per ml; L-α-phosphatidylethanolamine and L-α-phosphatidylcholine in a 3:1 ratio) were mixed with the membrane vesicles (1 mg of protein/10 mg of phospholipid) in 50 mM potassium piperazine-*N,N'*-bis(2-ethanesulfonic acid) (pH 7.0) supplemented with 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, and 1 mM dithiothreitol (buffer A). After freezing in liquid nitrogen and slow thawing at room temperature, the mixture (0.5- to 1.0-ml portions) was sonicated at 4°C for 4 s with a macrotip at an output of 4 μm (peak to peak). For loading of the hybrid membranes with PEP (10 mM), enzyme I (0.9 μM), and HPr (15 μM), these compounds were added to the proteoliposome/vesicle mixture prior to freezing. To remove the external enzymes and PEP, the hybrid membranes were washed twice by centrifugation (45 min at 185,000 × *g*; 4°C) and, subsequently, resuspended in buffer A to a final protein concentration of 3–5 mg/ml.

Transport Assays. Δp-driven lactose uptake and equilibrium exchange of lactose in intact cells of *E. coli* were carried out as described (8). Uptake of sugars in the hybrid membranes was performed in the presence or absence of the electron donor system ascorbate/KOH/*N,N,N',N'*-tetramethylphenylenediamine (TMPD)/cytochrome *c* (horse heart) and oxygen as described (7), except that the medium composition was changed to that of buffer A.

Immunoblotting. The amount of wild-type LacS and LacS(Δ160) proteins in the membrane was estimated by immunoblotting with antibodies directed against the synthetic peptides N-Met-Glu-Lys-Ser-Lys-Gly-Gln-Met-Lys-Ser-Arg-Leu-Ser (N terminus of LacS) and N-Cys-Glu-Lys-Val-Glu-Ala-Leu-Ser-Glu-Val-Ile-Thr-Phe-Lys-Lys-Gly-Glu-C-terminal acid (C terminus). The proteins of right-side-out membrane vesicles were separated by SDS/12.5% PAGE and transferred to poly(vinylidene difluoride) (PVDF) membranes by semidry electrophoretic blotting. Processing of the PVDF membranes and immunoblot detection using the Western-Light chemiluminescence detection kit were performed as recommended by the manufacturer (Tropix, Bedford, MA).

Plasmid Constructions/DNA Manipulations. C-terminal deletion mutants of LacS were made by exonuclease III digestion from the 5′ protruding *Bam*HI restriction site of pSKE8 (Fig. 1; ref. 8). The deletions were made unidirectional by digesting the adjacent *Sac* I restriction site thereby creating a 4-base 3′ overhang. After S1 nuclease and Klenow fragment of DNA polymerase treatment, the DNA ends were ligated and the mixtures were transformed to *E. coli* HB101. All manipulations were carried out as described in the Erase-a-Base system manual (Promega). Plasmid DNA of individual transformants was isolated and digested with *Pvu* II to estimate the size of the deletions (see Fig. 1). Subsequently, the deletion endpoints were determined precisely by nucleotide sequencing of double-stranded plasmid DNA (10).

The *S. thermophilus* ST11(Δ*lacS*) deletion strain was constructed by integrating and resolving in wild-type ST11 a nonreplicating plasmid carrying flanking sequences of the *lacS* gene (13). For this purpose, plasmid pEKS8 (4) was digested with *Aat* II and *Kpn* I (see Fig. 1), blunt-ended by Klenow fragment treatment, and religated to obtain pPD1. The 2568-base *Eco*RI fragment of pPD1 was isolated and ligated into the unique *Eco*RI site of the integration vector pBM31 (13), which yields pPD2. *S. thermophilus* ST11 was transformed with pPD2 and the obtained erythromycin-resistance colonies were grown for ≈100 generations under nonselective conditions. Cells were plated on Belliker agar medium supplemented with sucrose and screened for erythromycin sensitivity, followed by screening for a Lac^- phenotype. The genotype of a number of putative *S. thermophilus* ST11(Δ*lacS*) isolates was verified by Southern hybridization (10). The construction of *S. thermophilus* ST11(Δ*lacZ*), transformation by electroporation of *S. thermophilus*, and isolation of plasmid and genomic DNA from this organism have been described (13).



Protein	Activity (%)
Wild-type	100
Δ53	83+/-3
Δ79	48+/-6
Δ106	67+/-9
Δ144	23+/-5
Δ147	41+/-5
Δ160	92+/-13
Δ197	3+/-2

FIG. 1. Lactose transport protein deletion mutants. The 4.1-kb *Eco*RI chromosomal DNA fragment of pSKE8, containing the *lacS* gene of *S. thermophilus* in the plasmid vector pBluescript II SK^+ are shown. Only relevant restriction sites are indicated. The *lacS* promoter is shown as P_S. The *lacS* gene is indicated by an arrow, and the wild-type and truncated proteins with various portions of the IIA domain are depicted as bars. Deletions correspond to the number of C-terminal amino acids that are missing. Activity refers to the initial rate of lactose uptake in DW2 cells carrying pSKE8 (wild type or deletion mutant). Lactose uptake was assayed at a substrate concentration of 50 μM in 100 mM potassium phosphate (pH 6.5) supplemented with 2 mM $MgSO_4$ and the electron donor D-lithium lactate (final concentration, 10 mM). The 100% value corresponds with an initial rate of 5.6 nmol per min per mg of protein. Assay temperature was 30°C.

For the expression of wild-type LacS in *S. thermophilus* ST11(Δ*lacS*), the 3784-bp *Eco*RI/*Dra* I fragment of pSKE8 (see Fig. 1) was ligated into the *E. coli*/*S. thermophilus* shuttle vector pGK13 (digested with *Eco*RI and *Eco*RV). The corresponding plasmid was named pGKGS8; pGKGS8(*lacS-H552R*) was made similarly using pSKE8(*lacS-H552R*) (8). The shuttle vector carrying the ΔIIA deletion gene was made by isolating the 3308-bp *Eco*RI/*Bss*HI fragment of pSKE8(*lacS*Δ*160*), filling the recessed 3′ terminus of the *Bss*HI site, and ligating the fragment into pGK13 digested with *Eco*RI and *Eco*RV. This plasmid was named pGKGS8(*lacS*Δ*160*).

Miscellaneous. The membrane potential (ΔΨ) was determined from the distribution of the lipophilic cation tetraphenylphosphonium (TPP^+) using a TPP^+-selective electrode as described (7, 14). Protein was measured by the method of Lowry *et al.* (15) in the presence of 0.5% SDS, using bovine serum albumin as a standard. Bovine heart cytochrome *c* oxidase was isolated according to described procedures (16). Enzyme I and HPr of *Bacillus subtilis* were purified as described (17).

RESULTS

LacS Deletion Mutants. To assess a possible functional interaction between the transport carrier and IIA domain of LacS, C-terminal deletions were made by exonuclease digestion of the 3′ region of the *lacS* gene. The corresponding C-terminal truncation mutants are shown in Fig. 1. Individual mutants in *E. coli* were tested for their ability to take up lactose in response to a protonmotive force (Δp) and to catalyze equilibrium exchange at a lactose concentration of 5 mM. The results of the Δp-driven uptake indicate that LacS mutants with C-terminal deletions up to 160 amino acids are still active and accumulate lactose against a concentration gradient inside

the cell (Fig. 1). The activities of the various mutants differed, most likely as a result of different expression levels of the truncated proteins. Importantly, however, the mutant Δ160 catalyzed lactose uptake (and accumulation) at a rate comparable to that of the wild-type protein. The mutant Δ197, which has a deletion that covers the entire IIA domain, the linker region, and approximately half of putative transmembrane helix XII, did not catalyze lactose transport. The relative exchange activities of the various mutants were comparable to the Δp-driven uptake activities (data not shown). Altogether, the results indicate that the IIA domain of LacS can be deleted without effect on the transport activity.

Construction of *lacS* Deletion Strain. In a previous report (8), we have shown that LacS can be phosphorylated *in vitro* by PEP and the general energy-coupling proteins of the *B. subtilis* PTS. Phosphorylation of LacS could not be detected with enzyme I and HPr isolated from *E. coli* (8), and, therefore, it is unlikely that PTS enzymes have affected the activity measurements in *E. coli*. To analyze how PEP-dependent enzyme I/HPr-mediated phosphorylation affects the lactose transport protein, the catalytic activities of wild-type LacS, LacS(H552R), and LacS(Δ160) were studied in *S. thermophilus*. For this purpose, it was essential to first inactivate the chromosomal *lacS* gene. Most of the coding region of the *lacS* gene was deleted from the chromosome of *S. thermophilus* ST11 by homologous recombination using flanking regions of the gene (Fig. 2). By Southern analysis of total genomic DNA, it was shown that the correct fragment of ≈1500 bp, containing most of *lacS*, was deleted. Fig. 3*A* shows that counterflow of the nonmetabolizable lactose analog methyl β-D-thiogalactopyranoside (TMG) was completely abolished in the Δ*lacS* mutant, whereas deletion of the gene encoding β-galactosidase (*lacZ*) had little effect on the uptake. Similar results were obtained when sucrose-energized (Δp driven) uptake was analyzed (data not shown). Not only uptake but also efflux down a concentration gradient and equilibrium exchange of TMG were totally lacking in the Δ*lacS* mutant (data not shown), indicating that the LacS protein is the only (secondary) galactoside transporting activity present in *S. thermophilus* ST11.

Complementation of *S. thermophilus* (Δ*lacS*) in trans. To express the LacS (wild type) and LacS mutant proteins in *S. thermophilus* ST11(Δ*lacS*), the corresponding genes were transferred from the ColE1-type plasmids pSKE8 and derivatives to a vector (pGK13) that replicates in the lactic acid bacterium. The constructed plasmids complemented the chromosomal deletion of *lacS* in trans but the transport activities varied (Fig. 3*B*). For instance, analysis of Δp-driven TMG uptake in sucrose-metabolizing cells yielded a lower activity for strain ST11(Δ*lacS*)/pGKGS8(wild-type *lacS*) than ST11(Δ*lacS*)/pGKGS8(*lacSΔ160*) (Fig. 3*B*; similar results were obtained when uptake of lactose down the concentration gradient was assayed), whereas TMG counterflow activity in energy-depleted cells of both strains was similar (data not shown). Western analysis of different membrane preparations

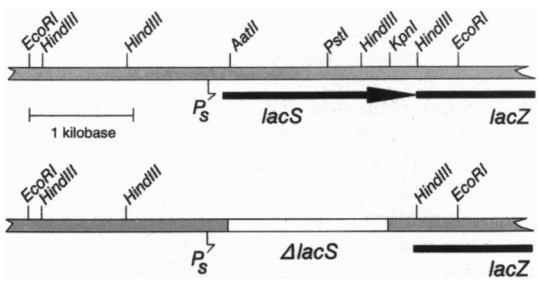


FIG. 2. Chromosomal deletion of *lacS* gene. Part of the genome of *S. thermophilus* ST11(wild type) and ST11(Δ*lacS*) is shown. The deletion of ST11(Δ*lacS*) corresponds with the 1505-bp *Aat* II/*Kpn* I fragment.

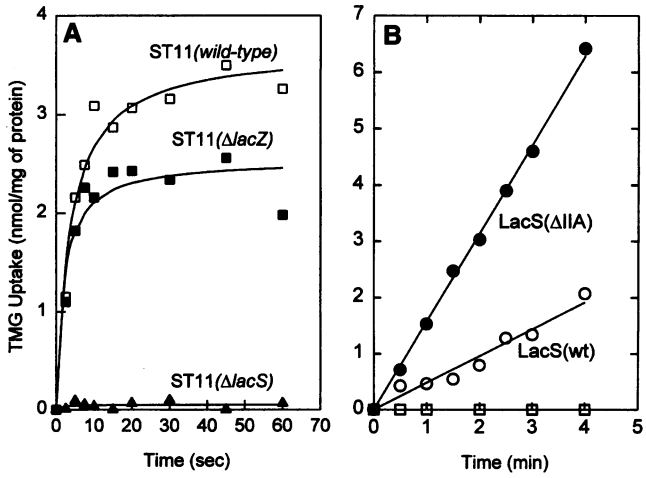


FIG. 3. Galactoside transport in *S. thermophilus* ST11, ST11(Δ*lacS*), and ST11(Δ*lacZ*) (*A*) and complementation in trans of the ST11(Δ*lacS*) strain (*B*). *S. thermophilus* cells were grown in Belliker broth with sucrose plus galactose. Cells were washed and resuspended in 50 mM potassium phosphate (pH 6.5) supplemented with 2 mM $MgSO_4$ (buffer B). For TMG counterflow (*A*), the cells were equilibrated with 2 mM TMG for 1 h at 42°C and, subsequently, concentrated by centrifugation. To initiate counterflow, the cells were diluted 1:250 into buffer B containing 50 μM [^{14}C]TMG. Final protein concentrations were 0.12–0.15 mg/ml. For sucrose-driven TMG uptake (*B*), the ST11(Δ*lacS*)/pGKGS8 (○) and ST11(Δ*lacS*)/pGKGS8(*lacSΔ160*) (●) cells were grown in the presence of lactose, washed, and resuspended in buffer B. To initiate the uptake, the cells were diluted to a final protein concentration of 0.21–0.29 mg/ml into buffer B containing 10 mM sucrose and 210 μM [^{14}C]TMG (circles). Uptake of [^{14}C]TMG was not observed in the absence of sucrose (squares). Assay temperature was 30°C.

expressing the wild-type and IIA-truncated protein indicated that the proteins were present in comparable amounts. As expected, the antibody directed against the C-terminal peptide detected only the wild-type LacS protein and not the IIA-truncated protein (Fig. 4). The antibody directed against the N-terminal peptide detected both proteins. The shift in mobility of LacS(Δ160) corresponds with the deletion of 160 amino acids.

Sucrose metabolism could have affected the LacS-mediated TMG uptake by the two strains differently—i.e., via PEP/enzyme I/HPr phosphorylation of LacS(wild type), which cannot take place with LacS(Δ160). Since phosphorylation of LacS is difficult to manipulate in intact cells, further studies on the role of PEP/enzyme I/HPr-dependent phosphorylation were carried out in artificial membranes.

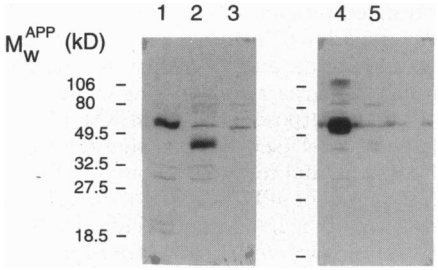


FIG. 4. Western blots of right-side-out membrane vesicles of *S. thermophilus* harboring LacS, LacS(Δ160), or no LacS protein. Corresponding strains were ST11/pGK13 (lanes 1 and 4), ST11(Δ*lacS*)/pGKGS8(*lacSΔ160*) (lanes 2 and 5), and ST11(Δ*lacS*)/pGK13 (lane 3). Blots were incubated with site-directed polyclonal anti-N-terminal (lanes 1–3) and anti-C-terminal (lanes 4 and 5) antibodies, respectively. Twenty micrograms of membrane protein was loaded in each lane.

Reconstitution of Phosphotransferase Activity. Membrane vesicles of *S. thermophilus* expressing wild-type LacS, LacS(H552R), and LacS(Δ160) were isolated and fused with proteoliposomes containing beef heart mitochrondrial cytochrome *c* oxidase as a protonmotive force-generating mechanism (7, 12). When appropriate, PEP plus enzyme I and HPr were incorporated in the hybrid membranes in order to effect phosphorylation of the LacS protein. To demonstrate that PEP-dependent enzyme I/HPr-mediated phosphoryl transfer does occur in the hybrid membranes, sucrose uptake by the PTS was assayed first. High levels of sucrose uptake were indeed observed ($label_{IN}/label_{OUT} \approx 1000$ after 5 min), and this transport activity was strictly dependent on PEP and enzyme I plus HPr (Fig. 5*A*). In the presence of a Δp, generated by oxidation of ascorbate/TMPD/cytochrome *c*, without the presence of PEP and/or the energy coupling proteins of the PTS, no uptake of sucrose was observed. The uptake of sucrose in the presence of PEP and enzyme I plus HPr was slightly inhibited by the Δp. These results indicate that sucrose–PTS activity can be reconstituted efficiently in hybrid membranes of *S. thermophilus* and that phosphoryl transfer from HPr to the IIA domain of LacS could be possible.

Effect of PEP-Dependent Enzyme I/HPr-Mediated Phosphorylation on LacS Activity. Lactose is taken up by the hybrid membranes and accumulation of the galactoside was strictly dependent on the presence of a Δp (Fig. 5*B*; $lactose_{IN}/lactose_{OUT} \approx 100$ after 5 min). In the presence of PEP and enzyme I plus HPr but in the absence of a Δp, accumulation of lactose was not observed, which is consistent with a lactose–H^+ symport mechanism. The Δp-driven uptake of lactose, however, was reduced by the presence of PEP and enzyme I plus HPr on the inside. The presence of PEP and the PTS enzymes on the outside was without effect on lactose transport, whereas the presence of PEP and enzyme I plus HPr on both sides of the membrane did not inhibit the transport of lactose further than when the compounds were present only on the inside (Fig. 5*B*). Coreconstitution of the lactose transport protein and cytochrome *c* oxidase with individual components of the PTS—i.e., PEP, enzyme I, or HPr—had no effect on Δp-driven lactose uptake (data not shown). From these results, it is tentatively concluded that PEP-dependent enzyme I/HPr-mediated phosphorylation of LacS inhibits transport activity. Control experiments indicated that this inhibition was not a consequence of a lowering of the Δp when PEP and the PTS phosphoryl transfer enzymes were present internally (data not shown). Also, reconstitution in the presence of HPr plus ATP, which could yield phosphoseryl HPr (17), did not affect lactose transport under the conditions used.

Table 1. Effect of PEP-dependent enzyme I and HPr-mediated phosphorylation on lactose transport by wild-type LacS, LacS(H552R), and LacS(Δ160)

Component(s)	Lactose uptake rate, nmol per min per mg of protein		
	LacS	LacS(H552R)	LacS(Δ160)
None	2.82 ± 0.15	2.36 ± 0.20	2.56 ± 0.14
HPr	2.61 ± 0.19	2.40 ± 0.17	2.36 ± 0.09
PEP + enzyme I + HPr	1.03 ± 0.24	2.66 ± 0.38	2.16 ± 0.10

Membrane vesicles of *S. thermophilus* ST11/pGK13 (wild type), ST11(Δ*lacS*)/pGKGS8(*lacSH552R*), and ST11(Δ*lacS*)/pGKGS8 (*lacSΔ160*) were fused with cytochrome *c* oxidase-containing liposomes in the absence or presence of PEP and/or PTS enzymes as indicated. Conditions were as specified in the legend to Fig. 5, except that the final concentration of [^{14}C]lactose was 6 μM. PTS components were present on the inside and outside. Initial rates were estimated from the uptake in time intervals up to 60 s; results are presented as means of two independent experiments.

Comparison of Wild-Type LacS, LacS(H552R), and IIA-Deletion Mutant. To obtain further evidence for the role of PEP-dependent enzyme I/HPr-mediated phosphorylation in lactose uptake, transport in hybrid membranes bearing LacS(wild type), LacS(H552R), and LacS(Δ160) was compared. The mean values for the transport rates of two independent experiments are presented in Table 1. The rate of lactose uptake by the wild-type protein was clearly reduced by the presence of PEP and enzyme I plus HPr, whereas uptake by the H552R and IIA-deletion mutants was not significantly affected. The presence of HPr alone inside the hybrid membranes had no effect on lactose uptake. This experiment provides strong evidence for the notion that phosphorylation of the conserved His-552 residue in the IIA domain lowers the activity of the LacS carrier domain.

DISCUSSION

In this study it has been shown that the wild-type lactose transport protein (LacS) and the IIA-deletion mutant (LacSΔ160) are equally competent in catalyzing galactoside–H^+ symport, equilibrium exchange, and galactoside counterflow. The wild-type LacS protein is affected by the presence of PEP plus the energy-coupling proteins enzyme I and HPr only when these compounds are present on the inner

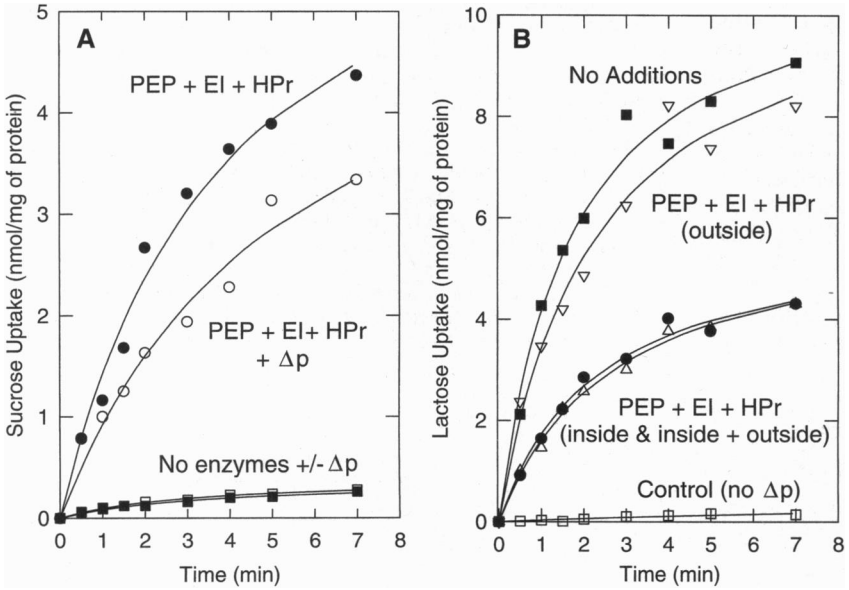


FIG. 5. Sucrose and lactose uptake in hybrid membranes of *S. thermophilus* ST11/pGK13. The presence or absence of PEP plus enzyme I and HPr is indicated. (*A*) PEP + enzyme I + HPr refers to the presence of these molecules on the inside. (*B*) Presence of PEP + enzyme I + HPr on the inside and/or outside is specified. For uptake, membranes were diluted 1:40 into buffer A supplemented with 10 mM ascorbate/KOH, 200 μM TMPD, and 20 μM horse heart cytochrome *c* (*A*, ○ and ■; *B*, ■, ▽, ●, and △) or without an electron donor system (*A*, ● and □; *B*, □). Final protein concentrations varied between 80 and 120 μg/ml. After 3 min of equilibration (preenergization) at 37°C, the transport assay was started by adding 2 μM [^{14}C]sucrose (*A*) or 9 μM [^{14}C]lactose (*B*).

surface of the membrane. PEP plus enzyme I and HPr do not affect the LacS(Δ160) protein, suggesting that phosphorylation of the IIA domain by HPr~P inhibits the activity of the carrier domain. Since the inhibition by PEP and the energy-coupling proteins of the PTS is also not observed with the LacS(H552R) mutant, we speculate that regulation takes place by means of phosphorylation of the conserved His-552 residue. Previous studies have shown that HPr~P of *B. subtilis* can phosphorylate LacS and that the site of phosphorylation most likely corresponds to His-552 (8). His-552 of LacS corresponds with His-90 of IIA^{Glc} in *E. coli*, which has also been shown to be the phosphoryl-accepting site (18, 19).

The regulation of activity of LacS is opposite that of dihydroxyacetone (glycerol) kinase of *Enterococcus faecalis*, which is activated by HPr~P-dependent phosphorylation (20). Whether regulation of the kinase occurs through phosphorylation of a IIA-like domain is unknown. These examples, however, indicate that PTS-mediated regulation of carbohydrate metabolism in these Gram-positive bacteria involves phosphorylation of the target proteins rather than interaction of the enzymes with a PTS component(s). Dephosphorylation of the lactose transport protein (LacS) and dihydroxyacetone (glycerol) kinase may occur in a backward reaction to HPr. The equivalent enzymes of enteric bacteria—i.e., the lactose transport protein (LacY) of *E. coli* and glycerol kinase (GlpK) of *E. coli* and *Salmonella typhimurium*—are inhibited by interaction with unphosphorylated IIA^{Glc} instead of HPr~P-dependent phosphorylation (1, 2).

The reason for the incomplete inhibition of lactose transport activity is not clear but could be due to the fact that only part of the transport molecule is phosphorylated. Note that phosphoryl transfer is mediated by a heterologous system—i.e., HPr from *B. subtilis* and LacS of *S. thermophilus*. It is unlikely that the incomplete inhibition of activity is caused by a random orientation of the LacS molecules in the hybrid membranes. First, the presence of PEP plus enzyme I and HPr on the outside does not affect lactose transport activity. Second, LacS is inhibited by the membrane-impermeable sulfhydryl reagent *p*-chloromercuribenzenesulfonic acid (pCMBS) only when the protein is reconstituted inside-out (B.P., unpublished results). From the insensitivity of LacS in the hybrid membranes toward pCMBS, we conclude that the protein is reconstituted with the IIA domain on the inner surface of the membrane.

In this study, it has also been shown that sucrose uptake in *S. thermophilus* is mediated by the PTS. The uptake of sucrose was slightly, but consistently, inhibited by a Δp (inside negative and alkaline relative to the outside). This inhibition could be due to an increased internal pH (21) or to a direct effect of the Δp on the PTS activity (1). It is important to emphasize that LacS does not have any PTS-mediated transport activity (see *Results*)—i.e., LacS cannot phosphorylate galactosides concomitant with transport.

The presence of a PTS for the uptake of sucrose suggests that this sugar could modulate the utilization of lactose by affecting the phosphorylation state of HPr. In contrast to *E. coli*, for instance, resting (washed) cells of *S. thermophilus* are rapidly depleted of metabolic energy, and PEP (and thus HPr~P) cannot be detected in these cells (unpublished results). Addition of a PTS sugar to these resting cells results in generation of a Δp, which drives LacS-mediated transport, but also in an increase in HPr~P, which is expected to slow down transport. The *in vivo* inhibition of transport is indeed observed when sucrose-energized transport of the nonmetabolizable lactose analog TMG in wild-type LacS and LacS(Δ160) is compared (Fig. 3*B*). In growing cells, however, the PTS sugar interferes with lactose utilization not only at the level of transport but also at the enzyme level in the glycolytic pathway (common pathway intermediates are formed from both sugars). Therefore, an activation of lactose transport by a lowering of the HPr~P concentration, possibly elicited by a PTS sugar when added to lactose-metabolizing cells, is not necessarily accompanied by a stimulation of lactose degradation. Since HPr~P is formed not only from the metabolism of a PTS sugar but also from lactose, we propose that regulation of lactose transport by HPr~P serves to prevent unbridled uptake of lactose (control of glycolysis) rather than to affect the hierarchy of sugar utilization. Such a mechanism allows lactose and sucrose to be cometabolized, which is indeed observed (unpublished results).

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